

CERTIFICATE OF MAILING (37 CFR 1.8a)

GRW

I hereby certify that this paper (along with any paper referred to as being transmitted therewith) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Date: January 28, 2008



Gene J. Yao
(Print Name)

(Signature)

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Group: 1641

Malte Buchholz, et al.

Serial No.: 10/696,487

Filed: October 29, 2003

For: **METHODS FOR DIAGNOSIS AND THERAPY OF PANCREATIC CANCER AND COMPOSITION
USEFUL THEREIN**

TRANSMITTAL OF CERTIFIED COPY

January 28, 2008

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Attached please find one certified copy of the foreign application from which priority is claimed for this case:

<u>Country</u>	<u>Application No.</u>	<u>Filing Date</u>
Europe	02024539.5	October 31, 2002

Respectfully submitted,

Gene J. Yao
Attorney for Applicant
Reg. No. 47,193
Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, New Jersey 07110
Phone: (973) 235-6993

GJY/ks
Enclosure
316450



Europäisches
Patentamt

European
Patent Office

Office européen
des brevets

Bescheinigung

Certificate

Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

02024539.5

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk



Anmeldung Nr:
Application no.: 02024539.5
Demande no:

Anmelde tag:
Date of filing: 31.10.02
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

F. HOFFMANN-LA ROCHE AG

4070 Basel
SUISSE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Methods for diagnosis and therapy of pancreatic cancer and composition useful
therein

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)
revendiquée(s)

Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

G01N33/48

An Anmelde tag benannte Vertragstaaten/Contracting states designated at date of
filing/Etats contractants désignés lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

Methods for diagnosis and therapy of pancreatic cancer and composition useful therein

The invention relates to the specific diagnosis of pancreatic cancer, compositions therefore and therapeutic methods for the treatment of pancreatic cancer.

Pancreatic cancer is a cancer type which is difficult to diagnose and to control and most of such cancers can grow very rapidly.

- 5 Neoplasms of the exocrine pancreas may arise from ductal, acinar and stromal cells. 80% of pancreatic carcinomas are derived from ductal epithelium. 60% of these tumors are located in the head of the pancreas, 10% in the tail and 30% are located in the body of the pancreas or are diffuse (Warshaw, A.L., and Fernandez-del, C.C., N. Engl. J. Med. 326 (1992) 4555-4565). Histologically, these tumors are graded as well as differentiated, moderately
- 10 differentiated and poorly differentiated. Some tumors are classified as adenosquamous, mucinous, undifferentiated or undifferentiated with osteoblast-like giant cells (Gibson, J.B., and Sabin, L.H., Histological typing of tumors of the liver, biliary tract and pancreas, WHO, Geneva, 1978). Since the disease is asymptomatic in its early stages, because of the lack of any test for early diagnosis and due to its aggressive character with respect to
- 15 metastasis locally and to visceral organs, this disease is associated with a dismal prognosis. Only 20% of the tumors are resectable and the survival benefit of approved chemotherapy regiments is rather poor (Kroep, J.R., et al., Ann. Oncol. 10, Suppl. 4 (1999) 234-238). Identification of new targets for early diagnosis of pancreatic tumors is therefore a challenge of paramount importance.
- 20 WO 02/08288 describes secreted and transmembrane polypeptides and nucleic acids encoding the same. Fig. 130 shows the polypeptide sequence of UKW.

Summary of the Invention

It was surprisingly found that nucleic acids coding for polypeptide UKW are overexpressed specifically in pancreatic tumor cells, whereas expression is considerably lower in normal

- 25 pancreatic cells or in tumor cells of other origin, e.g., breast cancer or colon cancer cells. Therefore, UKW is a valuable new target for specific diagnosis and therapy of pancreatic cancer.

The present invention therefore provides a method for determining the presence or absence of pancreatic cancer in a patient comprising

- (i) obtaining a biological sample from a patient;
- (ii) detecting in the sample an amount of nucleic acid encoding UKW or an amount of 5 polypeptide UKW; and
- (iii) comparing the amount of nucleic acid or polypeptide with a predetermined standard value indicating the decision line for tumor-induced or non-tumor-induced UKW expression or presence in the cell and therefrom determining the presence or absence of pancreatic cancer in the patient.

10

Preferably, the detection is performed by the use of a binding agent which binds to UKW nucleic acid or polypeptide. More preferably, the binding agent is a probe hybridizing under stringent conditions with UKW nucleic acid or an antibody, preferably a monoclonal antibody binding to UKW polypeptide, preferably to the extracellular domain.

15 Moreover, the present invention provides a process for determining whether or not a test sample of tissue or fluid of a patient contains pancreatic tumor cells or is derived from pancreatic tumor cells, wherein the test sample and a second sample originating from non-pancreatic-tumor cells from the same individual or a different individual of the same species are used, which process comprises the following steps:

20 (a) incubating each respective sample under stringent hybridization conditions with a nucleic acid probe which is selected from the group consisting of:

- (i) a nucleic acid sequence of SEQ ID NO: 1, or a fragment thereof;
- (ii) a nucleic acid sequence which is complementary to any nucleic acid sequence of (i);
- 25 (iii) a nucleic acid sequence which hybridizes under stringent conditions with the sequence of (i); and
- (iv) a nucleic acid sequence which hybridizes under stringent conditions with the sequence of (ii); and

30 (b) determining the approximate amount of hybridization of each respective sample with said probe, and

(c) comparing the approximate amount of hybridization of the test sample to an approximate amount of hybridization of said second sample to identify whether or not the test sample contains a greater amount of the specific nucleic acid or mixture of nucleic acids than does said second sample.

The invention also includes a method for the detection of pancreatic tumor, comprising

5 a) incubating a sample of a patient suspected of suffering from pancreatic cancer, selected from the group of body fluid, of cells, or of a cell extract or cell culture supernatants of said cells, whereby said sample contains nucleic acids with a nucleic acid probe which is selected from the group consisting of

(i) the nucleic acid shown in SEQ ID NO:1 or a nucleic acid which is complementary to said sequence, and

(ii) nucleic acids which hybridize with one of the nucleic acids from (i) and

10 b) detecting hybridization, preferably by means of a further binding partner of the nucleic acid of the sample and/or the nucleic acid probe or by X-ray radiography.

Both methods can also be performed by the use of antibodies, whereby the UKW polypeptide is detected by interaction between the antibody and the polypeptide.

15 The invention further provides a method for monitoring the progression of pancreatic cancer in the patient. In such a method, the amount of UKW nucleic acid or polypeptide in a biological sample (e.g., body fluids such as blood, cell lysates or a reverse transcript of an RNA sample) of a patient suffering from pancreatic cancer is determined at at least two different points in time and compared. From the change of the amount, information on the progression of said pancreatic cancer can be deduced.

20 The invention further comprises diagnostic kits comprising one or more oligonucleotide probes or primers for hybridization with UKW nucleic acid in a diagnostic assay using a sample obtained from a patient suffering from, or being suspected to have, pancreatic cancer.

25 The invention further comprises the use of antibodies against UKW polypeptide in a therapeutically effective amount in the treatment of pancreatic cancer. Preferably, the antibody is administered locally to the pancreas and especially preferably to the head of the pancreas.

30 In a further embodiment of the invention, the antibody against UKW polypeptide is administered to the patient in a therapeutically effective amount for the treatment of pancreatic cancer diseases and/or for preventing and/or inhibiting metastasis caused by pancreatic cancer diseases.

Detailed Description of the Invention:

As used herein, the term "UKW" means a nucleic acid encoding a polypeptide of SEQ ID NO:2, preferably the DNA sequence and the related mRNA sequence of SEQ ID NO:1 as well as the encoded polypeptide of SEQ ID NO:2. As UKW is a

5 transmembrane receptor protein, the polypeptide is of outstanding interest for diagnosis and as an epitope for antibody binding the extracellular domain of UKW polypeptide is preferred. Therefore, it is preferred to direct the nucleic acid sample and probes to this region and especially to parts thereof which have low homology with other genes and polypeptides.

10 The UKW receptor is a transmembrane protein composed of 373 amino acids with a signal sequence of 18 amino acids. The receptor consists of an extracellular domain of 215 amino acids, a transmembrane domain of 23 amino acids and a cytoplasmic domain of 117 amino acids. The extracellular domains displays two immunoglobulin C2-type folds composed of 93 amino acids and 72 amino acids.

15 The phrase "nucleic acid or polypeptide" as used throughout this application refers to a nucleic acid or polypeptide having a UKW activity which is substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or substantially free of chemical precursors or other chemicals when synthesized chemically. Such a nucleic acid is preferably free of sequences which naturally flank the nucleic acid
20 (i.e. sequences located at the 5' and the 3' ends of the nucleic acid) in the organism from which the nucleic acid is derived.

"Nucleic acid probes and primers for UKW" as used herein means nucleic acid fragments useful for the detection of UKW nucleic acids by hybridization methods. Hybridization techniques and conditions are well-known to one skilled in the art. Such hybridization
25 conditions are, for example, moderate stringent conditions including washing with a solution of 5 x SSC, 0.5% SDS, 1.0 mmol/l EDTA, pH 8.0, followed by hybridization at 50-60°C 5 x SSC overnight, washing at room temperature for 40 minutes with 2 x SSC containing 0.1% SDS and afterwards washing with 0.1 x SSC, 0.1% SDS at 50°C for 40 min with one change of fresh solution. It is also possible to use higher temperatures for
30 hybridization (e.g. 65-70°C) as high stringent hybridization conditions. The nucleic acid probes and primers usually consist of a UKW nucleic acid segment of at least about 50 contiguous positions most preferably of 200 to 300 nucleotides. The optimization of the probes and primers can be performed according to the state of the art. There exists

informatic software (http://www-genome.wi.mit.edu/genome_software/other/primer3.html) which is generally used for such probe and primer design.

For high selectivity it is preferred to use relatively low salt and/or high temperature conditions, for example, a salt concentration from about 0.02 mol/l to about 0.15 mol/l and 5 temperatures of from about 50°C to about 70°C.

UKW polypeptides can be identified in diagnostic assays using specific probes and primers. Usually such methods include amplifying the target sequence in the sample by amplification methods such as the PCR method. Quantitative detection can be performed by PCR techniques, preferably by the use of quantitative RT-PCR using, e.g., the 10 LightCycler® of Roche Diagnostics GmbH, DE.

In a preferred embodiment of the invention the coding nucleic acid of the sample is amplified before the test, for example by means of the known PCR technique. Usually a derivatized (labeled) nucleic acid probe is used within the framework of nucleic acid diagnostics. This probe is contacted with a denatured DNA, RNA or RT-DNA from the 15 sample which is bound to a carrier and in this process the temperature, ionic strength, pH and other buffer conditions are selected - depending on the length and composition of the nucleic acid probe and the resulting melting temperature of the expected hybrid - such that the labeled DNA or RNA can bind to homologous DNA or RNA (hybridization see also Wahl, G.M., et al., Proc. Natl. Acad. Sci. USA 76 (1979) 3683-3687). Suitable carriers are 20 membranes or carrier materials based on nitrocellulose (e.g., Schleicher and Schüll, BA 85, Amersham Hybond, C.), strengthened or bound nitrocellulose in powder form or nylon membranes derivatized with various functional groups (e.g., nitro groups) (e.g., Schleicher and Schüll, Nytran; NEN, Gene Screen; Amersham Hybond M.; Pall Biodyne).

To determine whether a test sample contains pancreatic tumor cells, the approximate 25 amount of hybridization of the nucleic acid with the target nucleic acid or nucleic acids is determined. The approximate amount of hybridization need not be determined quantitatively, although a quantitative determination is encompassed by the present invention. Typically, the approximate amount of hybridization is determined qualitatively, for example, by a sight inspection upon detecting hybridization. For example, if a gel is 30 used to resolve labelled nucleic acid which hybridizes to target nucleic acid in the sample, the resulting band can be inspected visually. When performing a hybridization of isolated nucleic acid which is free from pancreatic tumor cells from an individual of the same species, the same protocol is followed. One can compare the approximate amount of

hybridization in the test sample to the approximate amount of hybridization in the sample free from pancreatic tumor cells, to identify whether or not the test sample contains a greater amount of the target nucleic acid or nucleic acids than does the sample which is free from pancreatic tumor cells.

- 5 In a further method according to the invention no second sample is used. For the detection whether the expression of UKW gene is upregulated, the level of mRNA of UKW is compared with the level of mRNA of a standard gene (housekeeping gene (see, e.g., Shaper, N.L., et al., *J. Mammary Gland Biol. Neoplasia* 3 (1998) 315-324; Wu, Y.Y., and Rees, J.L., *Acta Derm. Venereol.* 80 (2000) 2-3) of the cell, preferably by RT-PCR.
- 10 As is shown in accordance with the present invention, the UKW nucleic acid is expressed in a greater amount in a pancreatic tumor sample than in a sample free from pancreatic tumor cells and/or in a greater amount than a housekeeping gene. A test sample containing pancreatic tumor cells will have a greater amount of the UKW nucleic acid than does a sample which is free from pancreatic tumor cells. To identify a test sample as containing 15 upregulated UKW nucleic acid, i.e., wherein the cells are pancreatic tumor cells or are tumor cells of a mammary carcinoma, it is preferable that the test sample have an approximate amount of UKW nucleic acid which is appreciably greater than the approximate amount in a sample free of pancreatic tumor cells. For example, a test sample having an upregulated UKW gene may have approximately 15- to approximately 60-fold 20 greater amount of UKW gene than a sample free of pancreatic tumor cells or an at least 3-fold greater amount of UKW mRNA than mRNA of a housekeeping gene like glycerolaldehyde-3-phosphate dehydrogenase (GAPDH) or porphobilinogen deaminase.

Methods of hybridization of a probe and a nucleic acid are known to a person skilled in the art and are described, for example, in WO 89/06698, EP-A 0 200 362, US 2,915,082, 25 EP-A 0 063 879, EP-A 0 173 251, EP-A 0 128 018.

Hybridizing DNA or RNA is then detected by incubating the carrier with an antibody or antibody fragment after thorough washing and saturation to prevent unspecific binding. The antibody or the antibody fragment is directed towards the substance incorporated during hybridization to the nucleic acid probe. The antibody is in turn labeled. However, it 30 is also possible to use a directly labeled DNA. After incubation with the antibodies it is washed again in order to only detect specifically bound antibody conjugates. The determination is then carried out according to known methods by means of the label on the antibody or the antibody fragment.

The detection of the expression can be carried out for example as:

- in situ hybridization with fixed whole cells, with fixed tissue smears,
- colony hybridization (cells) and plaque hybridization (phages and viruses),
- Southern hybridization (DNA detection),
- 5 - Northern hybridization (RNA detection),
- serum analysis (e.g., cell type analysis of cells in the serum by slot-blot analysis),
- after amplification (e.g., PCR technique).

10 The nucleic acids according to the invention are hence valuable markers in the diagnosis and characterization of pancreatic tumors.

According to the invention inhibitors for the expression of UKW (e.g., antibodies or antisense nucleotides) can be used to inhibit pancreatic tumor progression in vivo.

15 The invention further provides methods for identifying and isolation of antagonists of UKW or inhibitors for the expression of UKW (e.g., antibodies and antisense nucleotides). Such antagonists or inhibitors can be used to inhibit pancreatic tumor progression and cause massive apoptosis of pancreatic tumor cells in vivo.

20 According to the invention there are provided methods for identifying and isolation of UKW antagonists which have utility in the treatment of cancer. These methods include methods for modulating the expression of the polypeptides according to the invention, methods for identifying UKW antagonists which can selectively bind to the proteins according to the invention, and methods of identifying UKW antagonists which can modulate the activity of said polypeptides. The methods further include methods for modulating, preferably inhibiting, the transcription of UKW gene to mRNA. These methods can be conducted in vitro or in vivo and may make use of and establish cell lines 25 and transgenic animal models of the invention.

30 A UKW antagonist is defined as a substance or compound which decreases or inhibits the biological activity of UKW, a polypeptide and/or inhibits the transcription or translation of UKW gene. In general, screening procedures for UKW antagonists involve contacting candidate substances with host cells in which invasiveness is mediated by expression of UKW under conditions favorable for measuring UKW activity.

UKW activity may be measured in several ways. Typically, the activation is apparent by a change in cell physiology, such as increased mobility and invasiveness in vitro, or by a change in the differentiation state, or by a change in cell metabolism leading to an increase of proliferation.

- 5 The UKW polypeptides can be produced by recombinant means or synthetically. Non-glycosylated UKW polypeptide is obtained when it is produced recombinantly in prokaryotes. With the aid of the nucleic acid sequences provided by the invention it is possible to search for the UKW gene or its variants in genomes of any desired cells (e.g. apart from human cells, also in cells of other mammals), to identify these and to isolate the
- 10 desired gene coding for the UKW proteins. Such processes and suitable hybridization conditions are known to a person skilled in the art and are described, for example, by Sambrook et al., *Molecular Cloning: A Laboratory Manual* (1989) Cold Spring Harbor Laboratory Press, New York, USA, and Hames, B.D., Higgins, S.G., *Nucleic Acid Hybridisation - A Practical Approach* (1985) IRL Press, Oxford, England. In this case the
- 15 standard protocols described in these publications are usually used for the experiments.

With the aid of such nucleic acids coding for a UKW polypeptide, the polypeptide according to the invention can be obtained in a reproducible manner and in large amounts. For expression in prokaryotic or eukaryotic organisms, such as prokaryotic host cells or eukaryotic host cells, the nucleic acid is integrated into suitable expression vectors,

- 20 according to methods familiar to a person skilled in the art. Such an expression vector preferably contains a regulatable/inducible promoter. These recombinant vectors are then introduced for the expression into suitable host cells such as, e.g., *E. coli* as a prokaryotic host cell or *Saccharomyces cerevisiae*, Teratocarcinoma cell line PA-1, sc 9117 (Büttner, R., et al., *Mol. Cell. Biol.* 11 (1991) 3573-3583), insect cells, CHO or COS cells as eukaryotic
- 25 host cells and the transformed or transduced host cells are cultured under conditions which allow expression of the heterologous gene. The isolation of the protein can be carried out according to known methods from the host cell or from the culture supernatant of the host cell. Such methods are described for example by Ausubel I., Frederick M., *Current Protocols in Mol. Biol.* (1992), John Wiley and Sons, New York. Also in vitro reactivation
- 30 of the protein may be necessary if it is not found in soluble form in the cell culture.

UKW polypeptide or fragments thereof can be purified after recombinant production by affinity chromatography using known protein purification techniques, including immunoprecipitation, gel filtration, ion exchange chromatography, chromatofocussing,

isoelectric focussing, selective precipitation, electrophoresis, or the like and can be used for the generation of antibodies against UKW.

The invention further comprises recombinant expression vectors which are suitable for the expression of UKW, recombinant host cells transfected with such expression vectors, as

5 well as a process for the recombinant production of a protein which is encoded by the UKW gene.

Antibodies against UKW can be produced according to the methods known in the state of the art. For example, monoclonal or polyclonal antibodies can be produced using a polypeptide comprising about 10 to 100 amino acids of the UKW polypeptide sequence.

10 Suitable polypeptides derived from UKW include preferably amino acids from the extracellular domain, preferably amino acids 70-80, 99-113, 120-140 and 167-182.

The resulting antibodies can be screened for the ability to bind to UKW using standard techniques such as enzyme-linked immunoabsorbent assays. Methods for identifying antigen epitopes and for the production of antibodies are described, for example, in Mole,

15 "Epitope Mapping", In: Methods in Molecular Biology, Vol. 10, Manson (ed.), pages 105-116, The Humana Press, Inc., 1992; Price, "Production and Characterization of Synthetic Peptide-Derived Antibodies", In: Monoclonal Antibodies: Production, Engineering, and Clinical Application, Ritter and Ladyman (eds.), pp. 60-84, Cambridge University Press, 1995; Morris (ed.), Epitope Mapping Protocols 25, Humane Press, Inc., 1996; and Coligan
20 et al. (eds.), Current Protocols in Immunology, pp. 9.3.1-9.3.5 and pp. 9.4.1-9.4.11, John Wiley & Sons, 1997.

Antibodies which are useful according to the invention, especially for therapeutic purposes, can be identified by reducing the proliferation and invasive potential of pancreatic tumor cells. For this purpose, pancreatic tumor cells or a pancreatic tumor cell line, preferably cell

25 line SUIT-2 007 are treated with an antibody against UKW and proliferation and invasive potential are measured by Cell Proliferation Reagent WST-1 (a tetrazolium salt reagent, Roche Diagnostics GmbH, DE) and Matrigel invasion assay (BDS Biosciences, www.bdbiosciences.com).

30 Anti-UKW antibodies can be derived from any animal species or are chimeric or humanized antibodies. Especially preferred are human antibodies. Human monoclonal antibodies are obtained, for example, from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. In this technique,

elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described, for example, by Green, L.L., et al., *Nat. Genet.* 7 (1994) 13-21; Lonberg, N., et al., *Nature* 368 (1994) 856-859; and Taylor, L.D., et al., *Int. Immun.* 6 (1994) 579-591.

Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography 10 with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography (see, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines et al., "Purification of Immunoglobulin G (IgG)", In: *Methods in Molecular Biology*, Vol. 10, pages 79-104, The Humana Press, Inc., 1992).

The antibodies can be used for immunoassays according to the invention. Detection can be 15 performed by contacting a biological sample with an antibody, and then contacting the biological sample with a detectably labeled molecule, which binds to the antibody. The antibody can be conjugated with avidin/streptavidin (or biotin) and the detectably labeled molecule can comprise biotin (or avidin/streptavidin). Numerous variations of this basic technique are well-known to those of skill in the art. Alternatively, an antibody can be 20 conjugated with a detectable label to form an immunoconjugate. Suitable detectable labels include, for example, a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label or colloidal gold. Methods of making and detecting such detectably-labeled immunoconjugates are well-known to those of ordinary skill in the art and are described in more detail below.

25 Preferably, the antibodies according to the invention can be used for the treatment of a patient suffering from a pancreatic tumor. The advantage of such a therapy with a pharmaceutical composition comprising an anti-UKW antibody can be demonstrated in an in vivo pancreas tumor model. Such a model is described by Alves, F., et al., *Pancreas* 23 (2001) 227-235. This in vivo model comprises an orthotopic transplant model for 30 pancreatic ductal adenocarcinoma in severe combined immunodeficient (SCID) mice.

Generally, the dosage of administered anti-UKW antibodies will vary depending upon such factors as the subject's age, weight, height, sex, general medical condition and previous medical history. As an illustration, anti-UKW antibodies compositions can be administered

at low protein doses, such as 20 to 100 milligrams 30 protein per dose, given once, or repeatedly. Alternatively, the antibodies can be administered in doses of 30 to 90 milligrams protein per dose, or 40 to 80 milligrams protein per dose, or 50 to 70 milligrams protein per dose.

- 5 Administration of antibody components to a subject can be preferably intravenous, intramuscular, by perfusion through a regional catheter, preferably directly or vicinal to the pancreas organ. The administration may be by continuous infusion or by single or multiple boluses.

A pharmaceutical composition comprising an anti-UKW antibody, can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic proteins are combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a 0 pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. See, for example, Gennaro (ed.), Remington's Pharmaceutical Sciences, 19th edition, Mack Publishing Company, 1995.

For purposes of therapy, anti-UKW antibodies and a pharmaceutically acceptable carrier are administered to a patient in a therapeutically effective amount. A combination of the antibody and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant.

A pharmaceutical composition comprising anti-UKW antibodies is preferably furnished in liquid injectable or infusible form.

The following examples, references, sequence listing and figures are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

Description of the Figures

Figure 1 Differential expression of UKW mRNA in pancreatic tumor cell lines SUIT-2 007 and SUIT-2 028 analyzed by Northern blot analysis. (a: pancreatic tumor cell line SUIT-2 007; b: pancreatic tumor cell line SUIT-2 028)

Figure 2 Multiple Tissue Expression (MTETM) Array (Clontech, Palo Alto, CA, USA). This array contains normalized loadings of poly A⁺-RNA from 76 different tissues as well as control RNA's and DNA's and enables to screen for the presence and relative abundance of a target mRNA in a broad spectrum of fetal and adult tissues. H6 corresponds to 1 µg total RNA from pancreatic tumor cell line SUIT-2 007. The code is revealed below.

Figure 3 Relative expression (Taqman[®]-PCR) of new mRNA in samples derived from adenocarcinomas of the pancreas (PaCa), chronic pancreatitis (ChronPa) and normal pancreatic tissues (NorPA).

Figure 4 Relative expression of UKW mRNA in invasive and non-invasive pancreatic tumor cell lines.

Figure 5 Relative expression of UKW mRNA in invasive and non-invasive mammary tumor cell lines.

Pancreatic tumor cell lines:

- Suit-2 007¹
- MiaPaca-2, ATCC CRL 1420
- AsPC1, ATCC CRL 1682
- BxPC-3, ATCC CRL 1687
- Capan-1, ATCC HTB79
- IMIM-PC-1³
- IMIM-PC-2³
- Panc-1, ATCC CRL 1469
- Suit-2 028¹
- Capan-2, ATCC HTB80

Patu 8902²

Patu 8988s²

Patu 8988t²

Mammary tumor cell lines:

BT-549, ATCC HTB122
Hs578T, ATCC HTB126
MCF-10A, ATCC CRL 10317
MCF-12A, ATCC CRL 10782
MDA-MB-436⁴
MDA-MB-231, ATCC 45518
MDA-MB-435, ATCC 45526
MDA-MB-157, ATCC HTB24
BT-20, ATCC HTB19
BT-483, ATCC HTB121
CAMA-1, ATCC HTB-21
Du4475, DSM ACC427
MCF-7, ATCC HTB22
MDA-MB-175, ATCC 45516
MDA-MB-361, ATCC HTB27
MDA-MB-453, DSM ACC65
SK-BR-3, ATCC 45520
T47D, ATCC HTB133
UCAA-812⁴
ZR-75-1, ATCC CRL 1500
ZR-75-30, ATCC CRL 1504

¹ Taniguchi, S., et al., Clin. Exp. Metastasis 10 (1992) 259-266

² Elsasser, H.P., et al., Virchows Arch. B Cell Pathol. Incl. Mol. Pathol. 64 (1993) 201-207

5 ³ Wenger, C., et al., Oncogene 18 (1999) 1073-1080

⁴ Tong, D., et al., Breast Cancer Res. Treat. 56 (1999) 91-97

Example 1

Materials and Methods:

Northern blot:

10 µg of total RNA from SUIT-2 007 and SUIT-2 028 cell lines were loaded side by side on
5 a denaturing 1% agarose formaldehyde gel and size-separated by electrophoresis. Blotting to BrightStar-Plus™ positively charged nylon membrane was performed by capillary downward transfer. After UV-crosslinking (Stratagene UV Stratalinker 2400) the blot was hybridized. The RT-PCR product was labeled with α -[³²P]dATP to a specific activity of 2x10⁹ cpm/µg using the Strip-EZ™ DNA Kit (Ambion Inc., Austin, Texas). Pre-
10 hybridization (30 min) and hybridization (over-night) with the radioactive probe was performed in ExpressHyb™ Hybridization Solution (Clontech, Palo Alto, CA, USA) at 68°C. The membrane was washed in solution 1 (2x SSC, 0.05% SDS) at room temperature for 30-40 min with continuous agitation and several replacements of the wash solution 1 followed by a washing step with solution 2 (0.1 x SSC, 0.1% SDS) at 50°C for 40 min with
15 one change of fresh solution. The membrane was then exposed to Cronex, Medical X-Ray Films (Sterling Diagnostic Imaging Inc., USA) at -70°C for 2 h. Equal loading and transfer of mRNA to the membrane was assessed by rehybridizing the blot with α -[³²P]dATP-labeled GAPDH cDNA probe.

Multiple tissue expression array (MTE™)

20 The blot was hybridized with an α -[³²P]dATP labeled probe derived from UKW cDNA according to the instructions of the manufacturer and exposed to X-ray film at -70°C for 62 h.

Taqman®-PCR

Real-time quantitative PCRs were performed with the TaqMan® technology and the ABI
25 PRISM 7700 apparatus (Applied Biosystems, Foster City, CA). 10 µg total RNA isolated from frozen adenocarcinomas of the pancreas, chronic pancreatitis and normal pancreatic tissues were used for reverse transcriptase reactions in a volume of 20 µl. The PCRs were then carried out by mixing 200 ng cDNA with 4µl of 10x SYBR-Green buffer, 3 mM MgCl₂, 1 mM dNTDs, 0.2 units Uracil-N Glycosylase, 1 unit AmpliTaq Gold, 4µl primer mix (300
30 nM each primer: forward 5' TTCTCTTGACAGGTTCTGGGC3' (SEQ ID NO:3); reverse 5'GGTTGGAACCACTAGGGCCTC3') (SEQ ID NO:4) in a final volume of 40 µl. PCR

primers were designed to generate a DNA fragment of 50 bp using the Primer Express Software (PE Biosystems, CA, USA). The amplification cycles were as follows: 2 min at 50°C followed by 10 min at 95°C and 40 amplification cycles (95°C for 15 sec and 60°C for 60 sec). These experiments were performed twice. The results were calculated by subtraction of gene UKW and housekeeping gene xs13 expression levels for each sample. Each value was divided by the averaged steady-state level of UKW mRNA of three healthy tissues. Ratios were squared and a reciprocal value was formed. Primers: xs13for: 5'AGATCCGCATGTCCCTTC3' (SEQ ID NO:5); xs13rev: 5'CCTTGCGCATCATGGTGTT3' (SEQ ID NO:6).

10 LightCycler-PCR

LightCycler-PCR was performed with a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany) in LightCycler capillaries using a commercially available master mix containing Taq DNA polymerase, SYBR-Green I, deoxyribonucleoside triphosphates (LightCycler DNA master SYBR-Green I, Roche Molecular Biochemicals). After addition of primers (forward 5'CCCCAGGAGTTATGCTTGG3' (SEQ ID NO:7); reverse 5'GCCTGGATACCACACTACCAG3' (SEQ ID NO:8); final concentration: 0.5 µM), MgCl₂ (3 mM) and template DNA to the master mix, 37 cycles of denaturation (95°C for 1 sec), annealing (58°C for 5 sec) and extension (72°C for 8 sec) were performed. All temperature transition rates were set to 20°C per sec. After completion of PCR amplification, melting curve analysis was performed. For this procedure, PCR products were denatured at 95°C, annealed at 65°C, and gradually heated to 95°C. SYBR-Green I fluorescence was monitored stepwise every 0.1°C. The melting curve was analyzed by visual inspection, amplified UKW gene rearranged structures were melting at 85–88°C. To control for primer dimer formation a control without template DNA ('water control') was included in each experiment. A small peak was usually visible at 78°C, which could be discriminated from the peak caused by the specific amplification product at 85–88°C. Calibration curve for calnexin was generated using serial dilutions (1:10, 1:50 and 1:80) of cDNA from pancreatic cancer cell line Suit-2 007. The relative amounts of UKW cDNA and calnexin were determined based on a calibration curve. Relative expression of gene UKW was calculated as a normalized value. This was generated by dividing the steady-state levels of gene UKW and calnexin for each sample. Calnexin forward primer 5'ATTGTCAGATCGTTATTGC3' (SEQ ID NO:9); reverse primer 5'ATGGAACAGGTAACCAGCAT3' (SEQ ID NO:10).

Example 2

Production of antibodies against UKW polypeptide

For immunization the peptides aa 167-182 and aa 306-320 (SEQ ID NO:2) were synthesized and coupled to a carrier molecule. Immunization of two rabbits with the mix
5 of the peptides.

Standard immunization protocol (for rabbits):

Day 0: Taking of pre-immune serum followed by the first immunization

Day 14: Second immunization

Day 28: Third immunization

10 Day 38: Blood sampling

Day 56: Fourth immunization

Day 66: Blood sampling

Day 80: Complete bleeding

15 The antiserum recovered was purified by affinity chromatography.

List of References

Alves, F., et al., *Pancreas* 23 (2001) 227-235

Ausubel I., Frederick M., *Current Protocols in Mol. Biol.* (1992), John Wiley and Sons, New York

5 Baines et al., "Purification of Immunoglobulin G (IgG)", In: *Methods in Molecular Biology*, Vol. 10, pages 79-104, The Humana Press, Inc., 1992

Büttner, R., et al., *Mol. Cell. Biol.* 11 (1991) 3573-3583

Coligan et al. (eds.), *Current Protocols in Immunology*, pp. 9.3.1-9.3.5 and pp. 9.4.1-9.4.11, John Wiley & Sons, 1997

10 Elsasser, H.P., et al., *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* 64 (1993) 201-207
EP-A 0 063 879
EP-A 0 128 018
EP-A 0 173 251
EP-A 0 200 362

15 Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th edition, Mack Publishing Company, 1995
Gibson, J.B., and Sabin, L.H., *Histological typing of tumors of the liver, biliary tract and pancreas*, WHO, Geneva, 1978
Green, L.L., et al., *Nat. Genet.* 7 (1994) 13-21

20 Hames, B.D., Higgins, S.G., *Nucleic Acid Hybridisation - A Practical Approach* (1985) IRL Press, Oxford, England
Kroep, J.R., et al., *Ann. Oncol.* 10, Suppl. 4 (1999) 234-238
Lonberg, N., et al., *Nature* 368 (1994) 856-859
Mole, "Epitope Mapping", In: *Methods in Molecular Biology*, Vol. 10, Manson (ed.), pages 25 105-116, The Humana Press, Inc., 1992

Morris (ed.), *Epitope Mapping Protocols* 25, Humane Press, Inc., 1996
Price, "Production and Characterization of Synthetic Peptide-Derived Antibodies", In: *Monoclonal Antibodies: Production, Engineering, and Clinical Application*, Ritter and Ladyman (eds.), pp. 60-84, Cambridge University Press, 1995

30 Sambrook et al., *Molecular Cloning: A Laboratory Manual* (1989) Cold Spring Harbor Laboratory Press, New York, USA
Shaper, N.L., et al., *J. Mammary Gland Biol. Neoplasia* 3 (1998) 315-324
Taniguchi, S., et al., *Clin. Exp. Metastasis* 10 (1992) 259-266
Taylor, L.D., et al., *Int. Immun.* 6 (1994) 579-591

35 Tong, D., et al., *Breast Cancer Res. Treat.* 56 (1999) 91-97
US 2,915,082

Wahl, G.M., et al., Proc. Natl. Acad. Sci. USA 76 (1979) 3683-3687
Warshaw, A.L., and Fernandez-del, C.C., N. Engl. J. Med. 326 (1992) 4555-4565
Wenger, C., et al., Oncogene 18 (1999) 1073-1080
WO 02/08288
5 WO 89/06698
Wu, Y.Y., and Rees, J.L., Acta Derm. Venereol. 80 (2000) 2-3

Patent Claims

1. Method for determining the presence or absence of pancreatic cancer in a patient comprising
 - (i) obtaining a biological sample from a patient;
 - 5 (ii) detecting in the sample an amount of nucleic acid encoding UKW or an amount of polypeptide UKW; and
 - (iii) comparing the amount of nucleic acid or polypeptide with a predetermined standard value indicating the decision line for tumor-induced or non-tumor-induced UKW expression or presence in the cell and therefrom determining the presence or absence of pancreatic cancer in the patient.
- 10 2. Process for determining whether or not a test sample of tissue or fluid of a patient contains pancreatic tumor cells or is derived from pancreatic tumor cells, wherein the test sample and a second sample originating from non-pancreatic-tumor cells from the same individual or a different individual of the same species are used, which process comprises the following steps:
 - (a) incubating each respective sample under stringent hybridization conditions with a nucleic acid probe which is selected from the group consisting of:
 - 20 (i) a nucleic acid sequence of SEQ ID NO: 1, or a fragment thereof;
 - (ii) a nucleic acid sequence which is complementary to any nucleic acid sequence of (i);
 - (iii) a nucleic acid sequence which hybridizes under stringent conditions with the sequence of (i); and
 - 25 (iv) a nucleic acid sequence which hybridizes under stringent conditions with the sequence of (ii); and
 - (b) determining the approximate amount of hybridization of each respective sample with said probe, and
 - 30 (c) comparing the approximate amount of hybridization of the test sample to an approximate amount of hybridization of said second sample to identify whether or not the test sample contains a greater amount of the specific nucleic acid or mixture of nucleic acids than does said second sample.

3. Method for the detection of pancreatic tumor, comprising
 - a) incubating a sample of a patient suspected of suffering from pancreatic cancer, selected from the group of body fluid, of cells, or of a cell extract or cell culture supernatants of said cells, whereby said sample contains nucleic acids with a nucleic acid probe which is selected from the group consisting of
 - (i) the nucleic acid shown in SEQ ID NO:1 or a nucleic acid which is complementary to said sequence, and
 - (ii) nucleic acids which hybridize with one of the nucleic acids from (i) and
 - b) detecting hybridization, preferably by means of a further binding partner of the nucleic acid of the sample and/or the nucleic acid probe or by X-ray radiography.
- 15 4. Use of an antibody that binds to the polypeptide UKW of the sequence SEQ ID NO:2 in the manufacture of a composition for inhibiting the proliferation and/or invasive potential of pancreatic tumor cells.
5. Use according to claim 4, wherein the composition is administered to cell cultures in vitro.
- 20 6. Use according to claim 4, wherein the composition is a pharmaceutical composition and wherein the pharmaceutical composition is administered to a mammalian subject suffering from a pancreatic tumor.
7. Pharmaceutical composition containing an antibody against UKW having the polypeptide sequence SEQ ID NO:1 together with a pharmaceutically acceptable carrier.

Abstract

Polynucleotide and polypeptide UKW are specific for pancreatic tumors. Diagnosis of UKW is therefore valuable for diagnosis of pancreatic tumors. Antibodies against UKW are, besides their value in diagnosis, useful as therapeutic agents in the treatment of 5 pancreatic tumors.

EPO - Munich
67
31. Okt. 2002

~~Experiments~~ Fig. 1

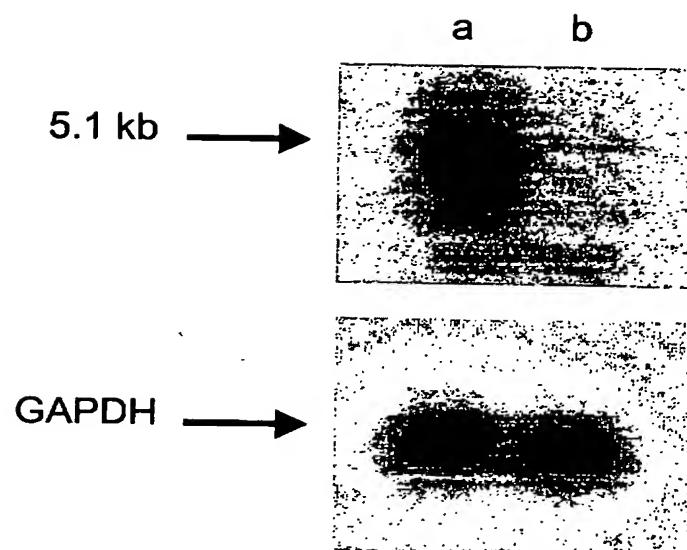
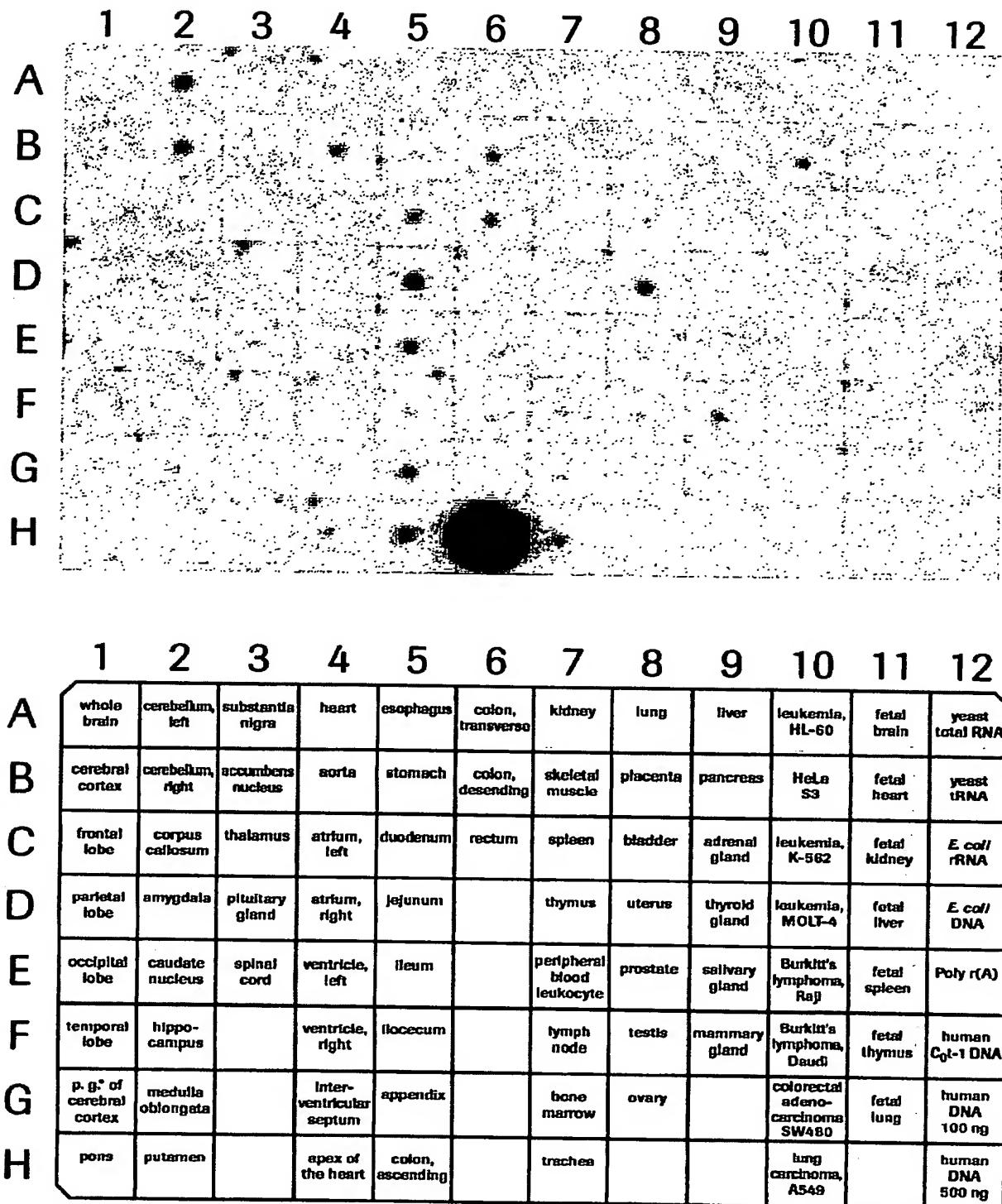


Fig. 2



* paracentral gyrus

Fig. 3

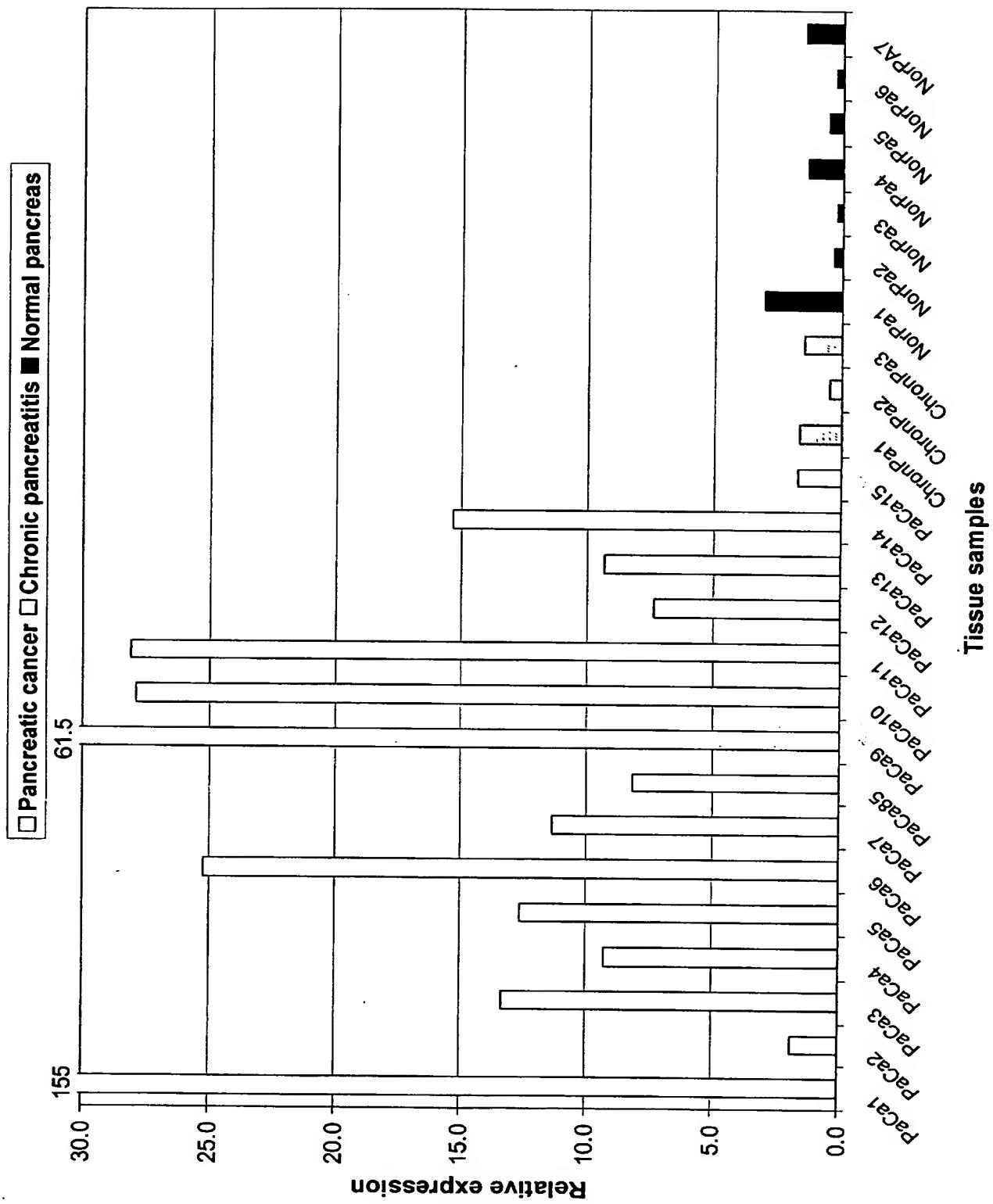


Fig. 4

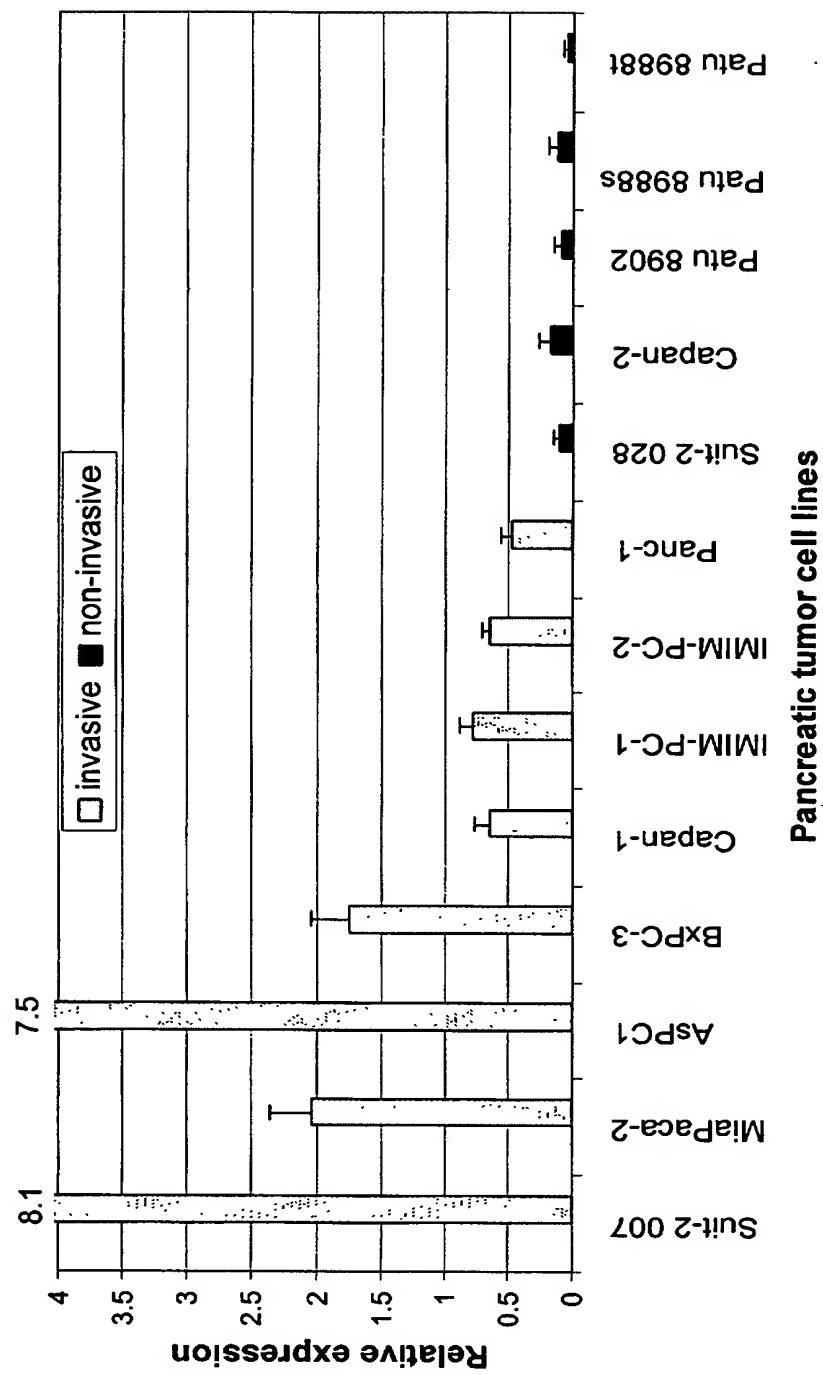
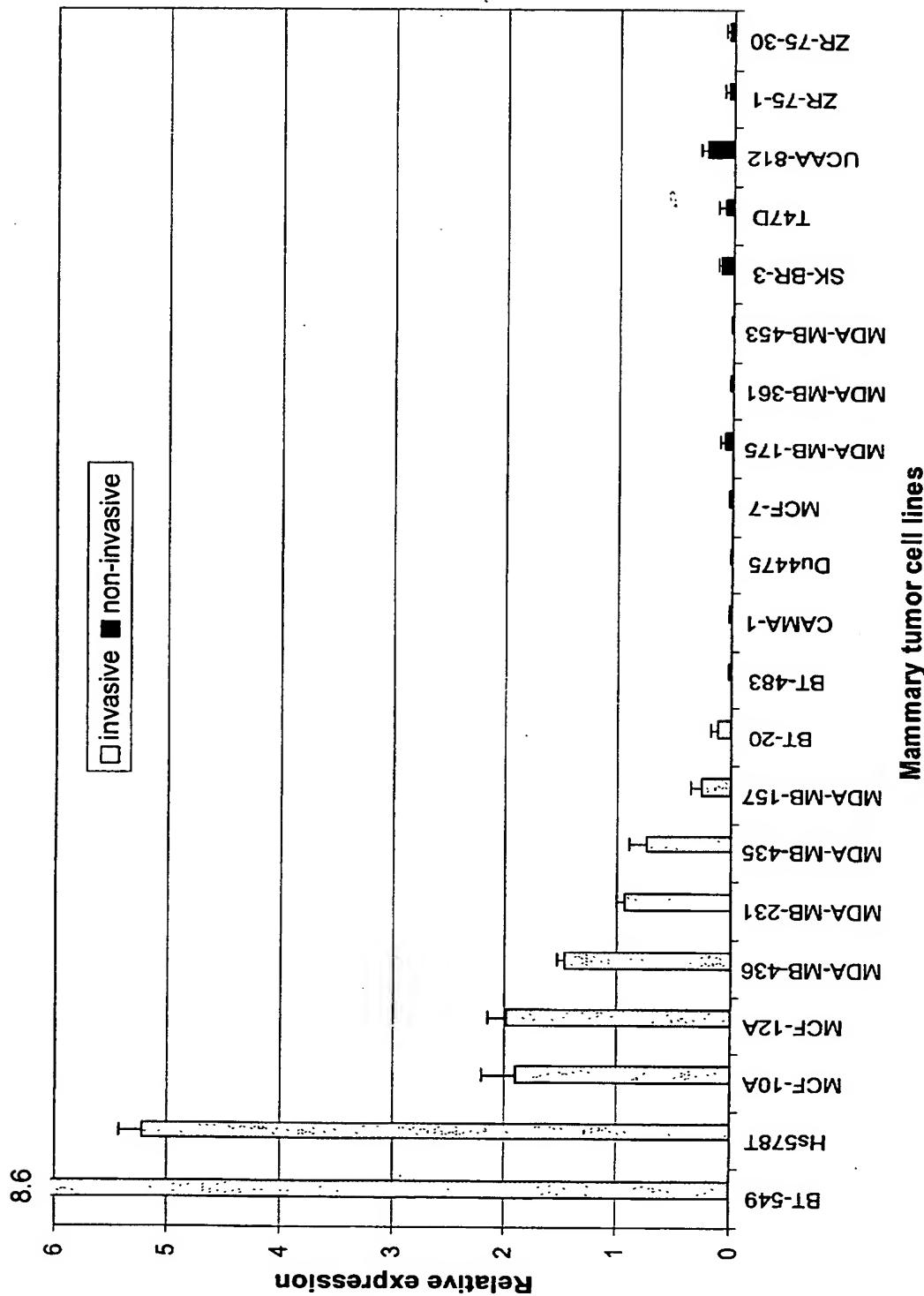


Fig. 5



SEQUENCE LISTING

<110> F. Hoffmann-La Roche AG
 5 <120> Methods for diagnosis and therapy of pancreatic cancer
 and composition useful therein
 <130> 21460 EP
 10 <140>
 <141>
 <160> 10
 15 <170> PatentIn Ver. 2.1
 <210> 1
 <211> 5120
 <212> DNA
 20 <213> Homo sapiens
 <220>
 <221> CDS
 <222> (366)..(1484)
 25 <400> 1
 ggttaggaggc aaccatgtgg ttccagctga atttttttt tccctctctc tttcttact 60
 ccttttctt tccaaacagg gaaaagtgtt ccacgaagcg gtaggcctt tccgcctcgc 120
 30 gttttcctcc ctgaccctgg tcccggctcc cgtccgggccc ccagctggtg gggcgagcgc 180
 cgggagccca tctgccccca gggcacggg gcgccccccc ggctcccgcc cggcacatgg 240
 35 ctgcagccac ctcgcgcgca ccccgaggcg cccgcggccag ctcgcccggag gtccgtcgga 300
 ggcgccccgc cgccccggag ccaagcagca gctgagcggg gaagcggccg cgtccgggga 360
 40 tcggg atg tcc ctc ctc ctt ctc ctc ttg cta gtt tcc tac tat gtt gga 410
 Met Ser Leu Leu Leu Leu Leu Leu Val Ser Tyr Tyr Val Gly
 1 5 10 15
 acc ttg ggg act cac act gag atc aag aga gtg gca gag gaa aag gtc 458
 Thr Leu Gly Thr His Thr Glu Ile Lys Arg Val Ala Glu Glu Lys Val
 45 20 25 30
 act ttg ccc tgc cac cat caa ctg ggg ctt cca gaa aaa gac act ctg 506
 Thr Leu Pro Cys His His Gln Leu Gly Leu Pro Glu Lys Asp Thr Leu
 35 40 45
 50 gat att gaa tgg ctg ctc acc gat aat gaa ggg aac caa aaa gtg gtg 554
 Asp Ile Glu Trp Leu Leu Thr Asp Asn Glu Gly Asn Gln Lys Val Val
 50 55 60

	atc act tac tcc agt cgt cat gtc tac aat aac ttg act gag gaa cag Ile Thr Tyr Ser Ser Arg His Val Tyr Asn Asn Leu Thr Glu Glu Gln 65 70 75	602
5	aag ggc cga gtg gcc ttt gct tcc aat ttc ctg gca gga gat gcc tcc Lys Gly Arg Val Ala Phe Ala Ser Asn Phe Leu Ala Gly Asp Ala Ser 80 85 90 95	650
10	ttg cag att gaa cct ctg aag ccc agt gat gag ggc cgg tac acc tgt Leu Gln Ile Glu Pro Leu Lys Pro Ser Asp Glu Gly Arg Tyr Thr Cys 100 105 110	698
15	aag gtt aag aat tca ggg cgc tac gtg tgg agc cat gtc atc tta aaa Lys Val Lys Asn Ser Gly Arg Tyr Val Trp Ser His Val Ile Leu Lys 115 120 125	746
	gtc tta gtg aga cca tcc aag ccc aag tgt gag ttg gaa gga gag ctg Val Leu Val Arg Pro Ser Lys Pro Lys Cys Glu Leu Glu Gly Glu Leu 130 135 140	794
20	aca gaa gga agt gac ctg act ttg cag tgt gag tca tcc tct ggc aca Thr Glu Gly Ser Asp Leu Thr Leu Gln Cys Glu Ser Ser Ser Gly Thr 145 150 155	842
25	gag ccc att gtg tat tac tgg cag cga atc cga gag aaa gag gga gag Glu Pro Ile Val Tyr Tyr Trp Gln Arg Ile Arg Glu Lys Glu Gly Glu 160 165 170 175	890
30	gat gaa cgt ctg cct ccc aaa tct agg att gac tac aac cac cct gga Asp Glu Arg Leu Pro Pro Lys Ser Arg Ile Asp Tyr Asn His Pro Gly 180 185 190	938
35	cga gtt ctg ctg cag aat ctt acc atg tcc tac tct gga ctg tac cag Arg Val Leu Leu Gln Asn Leu Thr Met Ser Tyr Ser Gly Leu Tyr Gln 195 200 205	986
	tgc aca gca ggc aac gaa gct ggg aag gaa agc tgt gtg gtg cga gta Cys Thr Ala Gly Asn Glu Ala Gly Lys Glu Ser Cys Val Val Arg Val 210 215 220	1034
40	act gta cag tat gta caa agc atc ggc atg gtt gca gga gca gtg aca Thr Val Gln Tyr Val Gln Ser Ile Gly Met Val Ala Gly Ala Val Thr 225 230 235	1082
45	ggc ata gtg gct gga gcc ctg ctg att ttc ctc ttg gtg tgg ctg cta Gly Ile Val Ala Gly Ala Leu Leu Ile Phe Leu Leu Val Trp Leu Leu 240 245 250 255	1130
50	atc cga agg aaa gac aaa gaa aga tat gag gaa gaa gag aga cct aat Ile Arg Arg Lys Asp Lys Glu Arg Tyr Glu Glu Glu Glu Arg Pro Asn 260 265 270	1178
55	gaa att cga gaa gat gct gaa gct cca aaa gcc cgt ctt gtg aaa ccc Glu Ile Arg Glu Asp Ala Glu Ala Pro Lys Ala Arg Leu Val Lys Pro 275 280 285	1226

agc tcc tct tcc tca ggc tct cg_g agc tca cgc tct ggt tct tcc tcc 1274
Ser Ser Ser Ser Ser Gly Ser Arg Ser Ser Arg Ser Gly Ser Ser Ser
290 295 300

5 act cgc tcc aca gca aat agt gcc tca cgc agc cag cg_g aca ctg tca 1322
Thr Arg Ser Thr Ala Asn Ser Ala Ser Arg Ser Gln Arg Thr Leu Ser
305 310 315

10 act gac gca gca ccc cag cca ggg ctg gcc acc cag gca tac agc cta 1370
Thr Asp Ala Ala Pro Gln Pro Gly Leu Ala Thr Gln Ala Tyr Ser Leu
320 325 330 335

15 gtg ggg cca gag gtg aga ggt tct gaa cca aag aaa gtc cac cat gct 1418
Val Gly Pro Glu Val Arg Gly Ser Glu Pro Lys Lys Val His His Ala
340 345 350

20 aat ctg acc aaa gca gaa acc aca ccc agc atg atc ccc agc cag agc 1466
Asn Leu Thr Lys Ala Glu Thr Thr Pro Ser Met Ile Pro Ser Gln Ser
355 360 365

25 aga gcc ttc caa acg gtc tgaattacaa tggacttgac tcccacgctt 1514
Arg Ala Phe Gln Thr Val
370

30 tccttaggagt cagggtcttt ggactcttct cgtcattgga gctcaagtca ccagccacac 1574
aaccagatga gaggtcatct aagtagcagt gagcattgca cggaacagat tcagatgagc 1634
atttccctta tacaatacca aacaagcaaa aggatgtaa ctgattcatc tgtaaaaagg 1694
catcttattg tgcctttaga ccagagtaag ggaaagcagg agtccaaatc tatttgtga 1754
ccaggacctg tggtgagaag gttggggaaa ggtgaggtga atatacctaa aacttttaat 1814

35 gtgggatatt ttgtatcagt gcttgattc acaatttca agaggaaatg ggatgctgtt 1874
tgtaaattt ctatgcattt ctgcaaactt attggattat tagttattca gacagtcaag 1934
40 cagaaccac agccttatta cacctgtcta caccatgtac tgagctaacc acttctaaga 1994
aactccaaaa aaggaaacat gtgtcttcta ttctgactta acttcatttgc tataaggtt 2054
tggatattaa ttcaagggg agttgaaata gtggagatg gagaagagtg aatgagttc 2114

45 tcccactcta tactaatctc actatttgc ttgagccaa aataactatg aaaggagaca 2174
aaaatttgc acaaaggatt gtgaagagct ttccatcttc atgatgttat gaggattgtt 2234
50 gacaaacatt agaaatataat aatggagcaa ttgtggattt cccctcaa at cagatgcctc 2294
taaggacttt cctgcttagat atttctggaa ggagaaaata caacatgtca tttatcaacg 2354
tccttagaaa gaattcttct agagaaaaag ggatcttagga atgctgaaag attacccaaac 2414
55 ataccattat agtctttct ttctgagaaa atgtgaaacc agaattgcaa gactgggtgg 2474

actagaaaagg gagatttagat cagtttctc ttaatatgtc aaggaaggta gccgggcatg 2534
gtgccaggca cctgtaggaa aatccagcag gtggaggtt cagttagcca agattatgcc 2594
5 attgcactcc agcctgggtg acaaagcaag actccatctc aaaaaaaaaa aaaaatcaag 2654
gaaggataaa aggaagttca gtattgtacc acacttggaa cttcctccat ttcttccatt 2714
ttagaaggat atgaacctgg aactttttagt gattctaagc cttaaactat caaaaagatc 2774
10 agggattgcc aatgcttcta atggcactgc aagtatatgc cataaccgtt ccctcctaaa 2834
agtgaaaaat gagagaaaatt cagtatttc ccaggctcag catccagaag tctagctctg 2894
15 ggctggaaga aaagggtact aatatttagg gaagagatga gaataagttag tgggtggcag 2954
gagagggctg agctagtgcc tgctaacatt ttagttgtat cttggaaaga tttagcaaaa 3014
ataactcacc aggatagctg ctgaagagtt gatgaatggg agaagaaaga tggggagaa 3074
20 ataaagaaaa cagcagcctg caatacaata acttgcctt ttaatagtt tgattactct 3134
tgataacctac agcacaaatg ctggacctga atcagctctt caaggaccct agcacaaatg 3194
25 tcaactgatc acctctggga gatgaaaaa cttttttttt tttgagacga agtctcgctc 3254
tggcccag gctggagtgc agtggcacca tctcagctca ctgcagcgtc cgcctttgg 3314
gttcaagtga ttctcctgcc ttgtcctcct gagtcgctgg tattacaggt gctgcccattc 3374
30 acacccagct aatttttggaa gttctgatag agacagggtt tctccatgtt ggccaggctt 3434
gtctcaagct cctgacactca agtgcacccatcc ccacccttgg cctccacag tgctgaaagc 3494
35 cactgcaccc ggctcagaat actttttttt ttgagatgca gtcttgctct catcacgcag 3554
gctggagtgc agtggcgtat ctcggctcac tgacacccatcc acctcccagtt ttcaagcgat 3614
tctccttcct cagccccca agtagcttgg attataggtt tgccacca cgtacagcta 3674
40 atttttgtat ttttagtaga gatggggttt cggccatgtt accaggctgg tctcaaaaacg 3734
cctgacactca ggtgatccac ccacccctggc cccacaaagt gctaggatta caggtgtgag 3794
45 ccaccatgcc cggccccagaa tacttttaa aagaagagca ggttagagga aaaaaaaaaa 3854
ttgatgctga atgtgggtat gaaagcatgt ttctaaaatg ggaagcagat gcttaaagag 3914
gaaagactaa tctgggattt tgccccattt ctctggttt tcactccat atttaattct 3974
50 cacaatcgatc tcgtcacata gtgaaaaaaaaa caaaattctt gtaaagtccc caggagttt 4034
tgcttgggtg aaagtttag cctgagtatt ttcttcctt aaaaaaggtg ggaaatgaga 4094
55 cattgaggaa ttaacatata aatgtctgct atgggtttaa gagaactggc gtattggaa 4154

tgcttcttac actaacactg ttcattgt aataataaaa ccccttactc taactacatt 4214
tttattcctc tggtagtgtg gtatccaggc aacatatcac ttctgctatg taattctaag 4274
5 aattctcatt tcttagagtac ctgagccaaa caaatacaca acggaagctg cagctgtatc 4334
atcaactagca atttgctcat cattatttac taccttgaa cctaaggttt cctgcctatg 4394
cttttggaaag caaaaatcag tctccttgc atgaaaaaga gccttagatt tttaaacatg 4454
10 ttagttacca gaatgctaaa ataccagttg attaccacaa ttatttgga aatctatcca 4514
taatggaagt ctacaacaaa cacataaaac agattacact aagagctgag aaattcaaag 4574
15 gaactgaaga ttctgagaga taaactgttc aagtcttagc aatgatactg cacttctt 4634
tgacagggttc tgggcttaag ttagaggccc tactggttcc aaaccatatt ccactgactt 4694
tgcaagtaaa ataaatttga ttctgaaata ggaaacaaaa aaaggagaaaa taaccgaata 4754
20 gtagaagaaaa aactgtttgt aggaagacga tgcagatgga atgatgtgga cattgagtaa 4814
ccatgtcaat aaaatata aaccaaactt aaatttgtga aataaggaag ttggcacctt 4874
25 tttgttaca gtgtataaaa acaatttcgg aactgctgtt gcaaaaagac atatatagtt 4934
ttgcttcctt ctgggtttaa gctgttata tttcagtttc agtttaact tctaagttgc 4994
cttgtaatttggactgtgtt tcagcatcac aaaaaccaaa tatttatttggatgcac 5054
30 gtatcagcaa ttaaaaata aacaagtaaa agtatactg taggagaagc tgaagctcaa 5114
aaaaaaa 5120
35
<210> 2
<211> 373
<212> PRT
<213> Homo sapiens
40
<400> 2
Met Ser Leu Leu Leu Leu Leu Leu Val Ser Tyr Tyr Val Gly Thr
1 5 10 15
45 Leu Gly Thr His Thr Glu Ile Lys Arg Val Ala Glu Glu Lys Val Thr
20 25 30
Leu Pro Cys His His Gln Leu Gly Leu Pro Glu Lys Asp Thr Leu Asp
35 40 45
50 Ile Glu Trp Leu Leu Thr Asp Asn Glu Gly Asn Gln Lys Val Val Ile
50 55 60
55 Thr Tyr Ser Ser Arg His Val Tyr Asn Asn Leu Thr Glu Glu Gln Lys
65 70 75 80

Gly Arg Val Ala Phe Ala Ser Asn Phe Leu Ala Gly Asp Ala Ser Leu
85 90 95

5 Gln Ile Glu Pro Leu Lys Pro Ser Asp Glu Gly Arg Tyr Thr Cys Lys
100 105 110

Val Lys Asn Ser Gly Arg Tyr Val Trp Ser His Val Ile Leu Lys Val
115 120 125

10 Leu Val Arg Pro Ser Lys Pro Lys Cys Glu Leu Glu Gly Glu Leu Thr
130 135 140

Glu Gly Ser Asp Leu Thr Leu Gln Cys Glu Ser Ser Ser Gly Thr Glu
145 150 155 160

15 Pro Ile Val Tyr Tyr Trp Gln Arg Ile Arg Glu Lys Glu Gly Glu Asp
165 170 175

Glu Arg Leu Pro Pro Lys Ser Arg Ile Asp Tyr Asn His Pro Gly Arg
20 180 185 190

Val Leu Leu Gln Asn Leu Thr Met Ser Tyr Ser Gly Leu Tyr Gln Cys
195 200 205

25 Thr Ala Gly Asn Glu Ala Gly Lys Glu Ser Cys Val Val Arg Val Thr
210 215 220

Val Gln Tyr Val Gln Ser Ile Gly Met Val Ala Gly Ala Val Thr Gly
225 230 235 240

30 Ile Val Ala Gly Ala Leu Leu Ile Phe Leu Leu Val Trp Leu Ile
245 250 255

Arg Arg Lys Asp Lys Glu Arg Tyr Glu Glu Glu Arg Pro Asn Glu
35 260 265 270

Ile Arg Glu Asp Ala Glu Ala Pro Lys Ala Arg Leu Val Lys Pro Ser
275 280 285

40 Ser Ser Ser Ser Gly Ser Arg Ser Ser Arg Ser Gly Ser Ser Ser Thr
290 295 300

Arg Ser Thr Ala Asn Ser Ala Ser Arg Ser Gln Arg Thr Leu Ser Thr
305 310 315 320

45 Asp Ala Ala Pro Gln Pro Gly Leu Ala Thr Gln Ala Tyr Ser Leu Val
325 330 335

Gly Pro Glu Val Arg Gly Ser Glu Pro Lys Lys Val His His Ala Asn
50 340 345 350

Leu Thr Lys Ala Glu Thr Thr Pro Ser Met Ile Pro Ser Gln Ser Arg
355 360 365

55 Ala Phe Gln Thr Val
370

5 <210> 3
<211> 22
<212> DNA
<213> Artificial Sequence

10 <220>
<223> Description of Artificial Sequence:forward primer
<400> 3
ttctctttga caggttctgg gc 22

15 <210> 4
<211> 21
<212> DNA
<213> Artificial Sequence

20 <220>
<223> Description of Artificial Sequence:reverse primer
<400> 4
25 gtttggaaacc agtagggcct c 21

30 <210> 5
<211> 18
<212> DNA
<213> Artificial Sequence

35 <220>
<223> Description of Artificial Sequence:xs13 forward
primer
<400> 5
agatccgcatttgc 18

40 <210> 6
<211> 19
<212> DNA
<213> Artificial Sequence

45 <220>
<223> Description of Artificial Sequence:xs13 reverse
primer
<400> 6
ccttgcgcatttgc 19

55 <210> 7
<211> 20
<212> DNA

<213> Artificial Sequence

<220>

5 <223> Description of Artificial Sequence:forward primer

<400> 7
ccccaggagt ttatgcttgg 20

10 <210> 8
<211> 21
<212> DNA
<213> Artificial Sequence

15 <220>
<223> Description of Artificial Sequence:reverse primer

<400> 8
gcctggatac cacactacca g 21
20

<210> 9
<211> 20
<212> DNA
25 <213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:forward primer

30 <400> 9
attgtcagat cgttcattgc 20

<210> 10
35 <211> 20
<212> DNA
<213> Artificial Sequence

<220>
40 <223> Description of Artificial Sequence:reverse primer

<400> 10
atggaacagg taaccagcat 20

45